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(57) Abstract

Apparatus for carrying out a biochemical assay, for example an immunoassay or nucleic acid hybridisation assay comprises, a hard polystyrene reaction surface adapted to bind a first biochemical ligand (e.g. a monoclonal antibody), a liquid absorbent wadding adjacent the reaction surface to absorb washing solution applied to the reaction surface, a filter matrix overlying the reaction surface in close contact therewith, for filtering a sample, and for retaining a second biochemical ligand in contact with the reaction surface, wherein the second biochemical ligand is capable of being specifically bound with the first biochemical ligand on the surface. The filter matrix is removeable to facilitate washing of the reaction surface, and the filter matrix comprises a labelled substance capable of a specific-binding reaction at the reaction surface during the assay.

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DEVICE AND METHOD FOR BIOCHEMICAL ASSAY

This invention relates to a method and apparatus for carrying out a biochemical assay, for example an immunoassay or DNA assay. In particular, the invention relates to such a method, which is easy and convenient to use, and does not require the use of sophisticated test equipment.

Biochemical assays, and in particular, immunoassays and so-called "DNA probes", have been carried out in very many different formats. A format which is very popular within the industry is to carry out immunoassay reactions in a well on a test plate, so as to bind to the wall of the well a labelled substance, in an amount which depends upon the amount of a test substance originally present in a sample. The usual method of detection in such assays is by spectrophotometric detection of a coloured reaction product, fluorescence, or, more recently, by electrochemical measurements, as disclosed, for example, in International Patent Application No. WO86/03837.

Such formats can give very good quantitative results, but generally require moderately complex apparatus for implementation and are not convenient to use for single test samples.

A number of proposals have been put forward for

simplified formats for immunoassays, in order to render them less complex to perform. For example, a method proposed by Elwing, H. and Nygren, H. (J. Immun. Methods, 31:101, 1979) discloses a method for the quantification of class-specific antibodies, in which substances to be detected spread out by diffusion in a gel, which is disclosed above a surface. After a period of time, the gel is removed, and the size of the region covered by the substance to be detected is measured, for example by incubation with an isotope, or enzyme-labelled antibody. The technique disclosed is a very insensitive one, and one which is unsuitable for analytes which are present in only minute quantities.

In recent times, a number of proposals have been made to carry out biochemical assays, and in particular immunoassays, in an absorbent matrix material, within which a specific binding species, such as an antibody, is localised. Examples of such systems are illustrated in U.S. 3888629, U.S. 4615983, and U.S. 4558012.

The nature of the surface of the materials employed in these types of matrixes enables a wide

wide range of non-specific binding reactions to take place in addition to the desired specific binding reactions. For the same reasons, it is difficult to wash excess immunological reagents from the matrix, after the specific binding reaction has taken place.

Various other test formats are disclosed in WO79/00044, EP 121385, EP 10456, WO80/02077, EP 170375, WO86/3837, EP 125139, and EP 201339. None of these references disclose a method which is particularly convenient in use, whilst being sufficiently sensitive for routine use in, say, a doctor's office.

WO85/02466 discloses a device intended to enable the rapid performance of immunoassays, for example enzyme immunoassays. The device includes a reaction site 35 on which an immunological reaction is carried out, and a number of absorbent pads (39, 41) which can be folded over to cover the reaction site 35, and which can contain various reagents for the immunoassay. The device can be folded such that the various absorbent pads can be brought into proximity with the test cell. In this device however, the absorbent pads 39, 41 are secured to segments of the device (31, 32) which are not fluid pervious. To carry out the assay, the samples are applied to the test surface 35, and the various pads 39, 41 are then

sequentially folded over to cover the sample on the test surface.

In accordance with the present invention, we have discovered that a device can be constructed in which a matrix for holding a ligand, usually the sample, in contact with a reaction surface may be combined with a filter, to enable solids present in the sample to be removed, and prevent them from coming into contact with the test surface. This is of particular value when the test apparatus is one designed for use in, for example, a doctor's office, in which it may be desirable to obtain a rapid determination, using a blood or urine sample for various clinical conditions, without the need for additional complex filtering mechanisms.

In accordance with the present invention, there is therefore provided apparatus for carrying out a biochemical assay comprising,

the solid support being a non-liquid-retaining, reaction surface adapted to bind a first biochemical ligand,

a liquid absorbent pad member adjacent the reaction surface adapted to absorb washing solution applied to the reaction surface,

a filter matrix adapted to contain a second biochemical ligand, capable of being specifically bound with the first biochemical ligand, and to retain

the said second ligand in contact with the reaction surface to overlie the reaction surface in close contact therewith.

The filter matrix not only provides filtering of the sample as it is added to the device, but also serves to contain the second by biochemical ligand (normally the sample), and retain it in contact with the reaction surface.

The reaction surface is preferably substantially flat, although it may, if desired be domed. The essential characteristic of the reaction surface is that it is non-absorbent, which reduces the amount of non-specific binding which takes place at its surface, and facilitates washing. Preferably the surface does not take the form of a well, which would retain reaction solution, and render washing difficult. The reaction surface is preferably formed of a non-fluid absorbent plastics material, for example polystyrene, PVC, cellulose acetate, polytetrafluroethylene, or "MYLAR". It is adapted to bind a first biochemical ligand, for example an antibody, an antigen, a protein such as haemoglobin, or a nucleotide sequence, such as a DNA or RNA fragment.

In a particularly preferred example, the first biochemical ligand is an antibody (particularly a monoclonal antibody), or a nucleotide sequence, and

the second biochemical ligand is constituted by the sample to be analysed (for example, an antigen or hapten recognised by the antibody, or a second nucleotide sequence respectively). In an alternative embodiment, the first biochemical ligand is derived from the sample and will bind spontaneously, but non-specifically to the reacion surface (for example, the first biochemical ligand may be haemoglobin) and the second biochemical ligand is a labelled substance which will bind specifically to the bound sample material (for example the second ligand is a labelled antibody).

The apparatus includes means for causing the filter matrix to overlie the reaction surface, in close contact therewith, thereby to enable a specific binding reaction to take place between the first and second biochemical ligand.

When the specific binding reaction between the first biochemical ligand, localised on the reaction surface, and the second biochemical ligand, retained in contact with the reaction surface by means of the filter matrix, has taken place, the filter matrix may preferably be removed, and the reaction surface may be readily washed, by applying a washing liquid thereto, the washing liquid being absorbed by the liquid absorbent pad located adjacent the reaction surface.

Means are provided for causing the filter matrix to overlie the reaction surface in close contact therewith.

For example, in one embodiment, the filter matrix may be permanently attached to the reaction surface,

and the reaction surface is washed following the specific binding reaction, by applying further quantities of washing solution to the exposed surface of the filter matrix. In this embodiment, if colour development is used to determine the result of the test the colour development must be viewed either through the filter matrix, or through the reaction surface.

In a much preferred embodiment however, the filter matrix is removable for example by virtue of being attached to a movable support, whereby it may be brought into and out of contact with the reaction surface.

The specific binding biochemical reaction takes place on the reaction surface, rather than within the matrix itself, and the presence of the filter matrix material maintains the sample in contact with the reaction surface. This results in substantially increased ease of production, facilitates the washing steps necessary during the biochemical reaction, and enables the production of a test with reduced noise caused by non-specific binding.

The removable support for the filter matrix may preferably be in the form of a cup-like member or frame, of which the absorbent filter matrix material forms the base, which may have a number of regions of filter matrix material. In this embodiment, the cup-like member may, in use, be positioned so that the filter matrix material overlies and is in contact with

the non-absorbent solid support, and solutions containing the material to be tested, washing solutions, and various reagents, may be poured into the cup and thereby brought into contact with the filter matrix material, whereby they contact the reaction surface after passage through the filter matrix material. In a preferred embodiment however, washing of the reaction surface is carried out after removal of the filter matrix.

In a particularly preferred embodiment of the invention, the filter matrix may contain a marker or label for the biochemical reaction, for example an enzyme conjugated to a specific binding substance, such as an antibody, an antigen, a hapten, or nucleotide fragment such as an DNA fragment. The marker is preferably provided in a dry form, whereby it may be activated, and caused to take part in a biochemical reaction, simply by wetting with an appropriate aqueous solution (normally the test sample and/or, if desired, a suitable buffer solution).

The method and apparatus of the invention may be utilised with any form of biochemical assay, for example an immunoassay, or so-called "DNA or RNA probe". For example, in a preferred embodiment, a monoclonal antibody may be immobilised to the reaction surface, and a conjugate of a different monoclonal

antibody with an enzyme, such as alkaline phosphatase, is provided in dry form in the filter matrix. Both monoclonal antibodies are such as will bind specifically with the antigenic substance it is desired to assay.

The substance to be assayed is then introduced onto the filter matrix, together with a suitable washing buffer if desired, and a sandwich is formed in which the enzyme becomes bound to the reaction surface.

The enzyme label may then be determined by any desired means, for example as described hereinafter.

In an alternative embodiment, the ligand bound to the reaction surface may be a polyclonal antibody, and the filter matrix may additionally comprise a substance consisting of a monoclonal antibody bound with a substance capable of binding with the polyclonal antibody.

In yet a further alternative, the reaction surface may be coated with a nucleotide fragment, for example single strand DNA, and the sample may be a second sequence generally a second DNA fragment. A solution containing the sample is introduced into a filter matrix in contact with the ligand support surface, and hybridisation takes place between the nucleotide fragments. Non-bound sample is removed

from the surface by a washing step, preferably after removal of the filter matrix. The amount of sample bound to the carrier may then be determined in a detection step, in which further hybridisation takes place between the bound sample, and a further nucleotide fragment, which has been labelled with a detectable marker. The detection step may also be any specific ligand anti-ligand reaction such as binding of an antibody to the hybridised nucleotide fragment sequence.

The detectable marker may be any of those commonly used in biochemical assays, but is preferably an enzyme. Detection thereafter takes place by any of the methods conventionally used in biochemical assays.

In yet a further alternative embodiment, the apparatus of the invention may be used to measure phenomena such as glycosylation in haemoglobin. It is known that glycosylated haemoglobin will bind to certain solids, in particular polystyrene in such a way as to render a specific part of the glycoacrylated haemoglobin to become susceptible to reaction with antibodies. Thus, in one embodiment, a sample which contains haemoglobin may be introduced onto the filter matrix, which is in contact with the non-absorbent reaction surface. The filter matrix may also comprise

an antibody which binds specifically with the specific part of the glyacrylated haemoglobin, the antibody having previously been labelled with a detectable marker, such as an enzyme. During the course of the assays, the haemoglobin becomes bound to the reaction surface, and the labelled antibody in turn binds to the haemoglobin, in dependence upon whether or not the haemoglobin is glycosylated. The detectable marker may be determined in the usual way.

The filter matrix material is able to remove various form of particulate matter from the sample, for example to remove blood cells from whole blood.

An additional removable filter may be provided, overlying the filter matrix maintained in contact with the reaction surface to provide filtering. Furthermore the filter material may be a two-layer matrix, of which one layer remains in contact with the reaction surface during the specific binding reaction, and the other layer provides the primary filtering function.

As indicated above, in a preferred embodiment of the invention, an enzyme label becomes bound to the reaction surface during the biochemical reaction, in an amount dependent upon the amount of the material under test present in the original sample. The bound enzyme may be determined in accordance with any

conventional method, for example using a method as disclosed in European Patent Application No. 60123, in which the enzyme is used to produce a trigger substance capable of taking part in a further biochemical reaction. In a preferred embodiment however, one or more reagents for effecting development to generate an observable change, in accordance with the amount of label bound to the reaction surface, (a developer) is associated with a moveable support material, which may also be an absorbent matrix, or may, for example, be a non-absorbent material such as an open mesh or other non-porous surface, and the apparatus is adapted to bring the support for the developer into close proximity with the reaction surface, in particular, to cause the developer support to replace the said filter matrix.

In a particularly preferred embodiment, the developer support forms the base of a cup or frame adapted to be brought into close proximity with the reaction surface.

In an alternative embodiment, the developer support may be hinged to the reaction surface substrate, whereby the developer support may be caused to overlie the reaction surface, by rotation about the hinge. The reagents contained in or on the developer

support may be, for example, substances capable of taking part in a cyclic biochemical reaction, for example the reversible interconversion of NADH and NAD, as disclosed in European Patent Specification No. 60123, and may be present in dry form.

The biochemical assay may be of any known type, for example a sandwich assay or a competition assay and the device in accordance with the invention preferably includes standard regions as well as sample regions for calibration purposes.

A number of preferred embodiments of the invention are illustrated in the accompanying drawings, in which:-

Figure 1 is an exploded view showing test apparatus in accordance with the invention,

Figure 2 is a schematic section of apparatus in accordance with the invention,

Figure 3 is a plan view of the apparatus of Figure 2,

Figure 4 is a schematic section of an alternative device in accordance with the invention,

Figure 5 is a plan view of the device in Figure 4,

Figure 6 is an example of a schematic reaction diagram for the device of Figures 4 and 5,

Figures 7 and 8 show further alternative devices

in accordance with the invention.

Figures 9 and 10 illustrate an alternative embodiment of the invention, incorporating electrodes,

Figures 11 and 12 are respectively a schematic section and plan of a cup incorporating electrodes,

Figures 13a and 13b are perspective and views respectively of a further alternative embodiment,

Figures 14a to 14a show yet a further alternative embodiment, and

Figures 15a and 15b show an electrode arrangement for use with a device as shown in Figures 14a to 14c.

Referring first to Figure 1, apparatus for carrying out an immunoassay comprises a hard inert polystyrene sheet, which serves as a reaction surface, and has dimensions approximately 10 cm x 3 cm. The polystyrene sheet 1 has a overlying layer 2 of an absorbent filter matrix. In the embodiment illustrated, the absorbent matrix was Whatman 541 Chromatography Paper, which is an inert cellulose matrix, to which conjugates of alkaline phosphatase do not bind non-specifically. A top layer 3 of the device includes a hole 4 defining a target area 5 of the absorbent filter matrix 2. A glass fibre filter 6 covers hole 4, and a label 7 is provided for indicia, to indicate the subject of the tests. Non-absorbent reaction surface 1 is provided with a coating of an antibody, shown schematically as 10. Coating of the polystyrene sheet with the antibody is carried out by any conventional method, for example as disclosed in

European Patent Application No. 132948. After being coated with the antibody, surface 1 is treated with bovine serum albumin (BSA) and other agents to serve as blocking agents for reducing non-specific binding as disclosed in European Patent Application No. 132948.

In an embodiment of the apparatus for determining levels of progesterone in a sample, for example for pregnancy testing, the antibody 10 may be anti-progesterone, and a conjugate of progesterone with alkaline phosphatase is provided in matrix layer 2.

When a sample to be analysed is introduced onto the target area 5 of filter matrix 2, the conjugate is dissolved, and binds specifically with antibody 10, in competition with the progesterone in the sample. Filter 6 reduces the amount of fatty and similar materials reaching matrix 2, and filter matrix 2 in turn reduces the amount of these substances reaching surface 1.

In an alternative embodiment, the antibody 10 may be a general antibody, and the matrix 2 may comprise a material including a monoclonal antibody, which is bound by the general antibody. This binding may take place either during the manufacture of the test device, or during the carrying out of the immunoassay.

The filter matrix 2 is essentially self-metering,

since reagents added to it spread out in a layer because of capillary action, leading to a constant concentration of reagents in the region of strip 1 immediately below target area 5. The cover sheet 3 is opaque, and circular hole 4 enables the result of the assay to be viewed. The various layers in the device may be bonded with adhesive, or, for example, affixed together mechanically, by means of rivets or the like. Cover layer 3 provides a clearly defined area which may be observed for colour development, so that it is not necessary to immobilise the antibody 10 and conjugate within any exact area.

Layer 6 may be discarded after addition of the sample, to reveal absorbent filter matrix layer 2. A suitable material for filter layer 6 may be glass fibre, such as Whatman GF/B, which acts as a depth filter to remove large particles.

A number of target areas 5 may be provided on the same test strip 1, simply by providing additional holes in the cover sheet 3. When the antibody 10 is a general antibody, each target area can be made specific for a particular analyte, either by incorporating in the matrix for that particular target region, or adding to the target region during the immunoassay process, specific antibodies. Hence, a single test strip could be used to assay urino-genital

sample for the presence of a number of antigens, for example Herpes in one target area, and Chlamydia in another. Additional target areas may be included, to provide positive and negative controls. In addition, since antibody 10 may be coated on both sides of sheet 1, additi nal target areas may be provided on the reverse side of sheet 1.

An immunoassay may be carried out utilising the apparatus of Figures 1 to 3, as follows. First, a sample containing the substance to be analysed is added to the assay target region 5. The sample can be added using a pipette or dropper, although great accuracy is not required, because the sample will spread through the absorbent filter matrix layer by capillary action to give an even concentration. The area wetted by the sample will ideally be larger than the assay target area 5 defined by the hole 4 in top sheet 3. The sample dissolves the dried conjugate, and a mixture of sample and conjugate will penetrate to the lower inert surface 1. It is preferable that the conjugate in the filter matrix dissolves only slowly upon addition of of sample, to avoid concentration of conjugate in the assay target area becoming unduly reduced, as the sample spreads laterally.

The device is incubated for two minutes at room

temperature. During this time, the sandwich will form between antibodies and antigen (if the assay is a sandwich assay), or the conjugate will become bound to the specific antibody or hapten or antigen (if the assay is a competition assay). If the antibody 10 bound to the plate 1 is a general antibody, the specific antibody may also become bound to the plate 1, during this part of the process.

The target area 5 is then washed, by dropping a washing buffer on to the assay target area from a dropper bottle. The washing solution may simply be a buffer solution, or, in a preferred embodiment, may contain substances to cause colour development, in the presence of the enzyme, together with an inhibitor, so that no reaction takes place during the washing step. In the washing step, excess fluid travels through the absorbent layer, and thus away from the assay target area 5.

In order to develop the colour in target region 5, an appropriate substance is then added, for example an enzyme substrate, or an anti-inhibitor. In a particular embodiment, the washing buffer may contain NADP, together with an enzyme inhibitor.

Optionally, a stopping solution may be added, to prevent further reaction after a predetermined time. This step may not be necessary, since the reaction may

stop of its own accord, due to evaporation of volatile reagents, or the further drying of the surface by capillary action. Thus, a colour develops in target region 5, to enable the presence or absence of the test substance in the sample to be determined.

An alternative embodiment of the device in accordance with the invention is illustrated in Figures 4 and 5. The device of Figures 4 and 5 comprises a hollow rectangular box 20, formed of a rigid plastics material (e.g. polystyrene). A compartment 21 may be separated from the remainder of the interior of the device by a dividing wall 22.

Two cup-like members 23 and 24 are threadedly received in holes provided in the upper surface of device 20.

The base 25 of cup 23 is formed of an absorbent water-permeable material, for example filter paper, or glass fibre. The base 26 of cup 24 may also be absorbent. Compartment 21 is provided with a filling of a water-absorbent material 28, for example cotton or synthetic wadding.

The lower surface 30 of device 20 is formed of, for example, polystyrene, and is provided with a layer of rubella antigen coated on its surface.

An absorbent filter matrix pad 32, for example of chromatography paper as disclosed above, contains a

conjugate of rubella antigen conjugated to an enzyme. The enzyme which is used in the enzyme conjugate, is preferably one which is capable of catalysing a reaction which results in the production of a trigger substance for a further reaction or series of chemical reactions, so as to enable the "amplification" of the response obtained, as disclosed in European Patent specification number 60123 and in U.K. Patent Application No. 84304328.2. In a particularly preferred embodiment, the enzyme is a phosphatase, for example alkaline phosphatase, and the development reactions consist of the reaction of alkaline phosphatase with nicotinamide dinucleotide phosphate ($NADP^+$) to produce nicotinamide dinucleotide (NAD^+) which acts as a trigger for the cyclic reaction of NAD^+ to NADH, in the present of ethanol, and an NAD^+-specific alcohol dehydrogenase. The reaction may produce either a colour change by reduction of a tetrazolium salt or an electrochemical signal by reduction at a platinum electrode as disclosed in International Patent Application No. WO 86/03837 or electron may be transferred to any suitable electrode for example via one or more electron carriers. The lower surface 26 of cup 24 contains the dry ingredients of an enzyme amplification system, as diclosed in European Patent Specifications Nos. 60123 and 132948. In use, a blood sample from a fingerprick is transferred, using a

capillary, to the absorbent base 25 of sample cup 23. The cup is preferably screwed in place to make firm contact between the filter pad 25 and the filter matrix pad 32 containing the enzyme conjugate. In an alternative embodiment, the cups 23 and 24 may be simply snap fits in device 20. Blood cells are retained by the filter material 25 and 32, and the plasma filters through absorbent filter matrix 32, to surface 30. Reagents may optionally be added at this stage, containing buffer or conjugate. If antibodies against rubella are present in the plasma, a sandwich is formed, as illustrated in Figure 6. The device is left for a period of from a few seconds up to twenty minutes or so, in order to allow the specific binding reaction to take place.

A washing solution is then added to the target area of the reaction surface 30, if desired, after removing the cup 23. As above, the washing solution may contain certain of the substances required for the development reaction. Alternatively, a separate developer solution may be added after the washing solution has drained away. The washing is effective to remove all free conjugate from the target area of the reaction surface 30. The sample filter cup 23 is then removed, if it has not been removed already, and it is replaced by cup 24. The moisture in the

conjugate layer dissolves the dry developer in the pad 26, and enables the development of colour, the intensity of the colour depending upon the amount of specific antibody in the blood sample. The presence or concentration of antibody in the blood sample can thus be estimated by visual inspection of the colour intensity.

The developer and the developer solution will generally consist of a substrate for the enzyme (i.e. the alkaline phosphatase) in a solution, which will generate a determinable signal by the action of the enzyme. For example, the complete developer solution may be as follows:-

0.1 mM NADPH or NADP

0.2 mg/ml alcohol dehydrogenase

0.15 mg/ml diaphorase

1mM p-iodonitrotetrazolium violet

4% ethanol

75 mM diethanolamine buffer, pH 9.0.

Other inert stabilizing components may be added.

The device may contain reagents and reaction sites to provide controls for the functionality of one or more of the reagents utilised. For example, part of the solid phase may contain an antibody against a portion of the conjugate such that a colour development will always be observed above that portion

of the solid phase if all reagents are functional.

Figures 7 and 8 illustrate a device somewhat similar to the device of WO 85/02466, in that it includes an absorbent pad 30 able to fold over to cover a reaction surface 31. The absorbent pad 30 is exposed on both its surfaces and is thus able to funtion as a filter for the sample material in use.

In an alternative aspect, the invention is concerned with apparatus of the type described, wherein the development reaction may be determined by an electrochemical change on a pair of electrodes, for example as disclosed in International Patent Application No. WO 86/03837. Figures 9 and 10 illustrate one form of the apparatus in accordance with the invention which might be operated in such a way. The apparatus of Figures 9 and 10 comprises a "MYLAR" sheet 10, to which are laminated a plurality of electrodes 61, 62, 63, 64, 65, 66.

Electrodes, 61, 62, 63, 64, 65 and 66 are arranged as segments of respective circles, electrodes 61 and 62 being, respectively, cathode and anode for measurements on region 52 of strip 51, and electrode 63 and 64, and 65 and 66 being, respectively, anode and cathode for regions 54, and 55 of strip 51. Electrodes 61 to 66 are connected via conductors 67 laminated to sheet 60, to an edge connector 68. Similar electrodes are connected for regions 56 and 57 of strip 51.

General or specific antibodies are coated onto strip 51, as described above, and an absorbent matrix containing alkaline phosphatase is then laminated to strip 51.

In use, the immunoassay is carried out in essentially the same manner as described above. However, determination of the bound enzyme is made not by observation of colour, but by measuring apparatus (not shown) connected to the said electrodes 61 to 66, via connector 68. The measuring apparatus includes means for applying a desired voltage to a pair of electrodes, for example to electrode 63 and 64, to measure the enzyme concentration in region 55, and for measuring the resulting current, which can be made to be dependent upon the amount of enzyme bound to region 55, as disclosed in International Patent Application No. WO 86/03837.

Figure 11 and Figure 12 illustrate a further alternative arrangement in which measurements can be made electrochemically. In this embodiment, electrodes 71 and 72 are laminated to the base 73 of a cup 74, generally similar to the cup 24 of Figure 4. The base portion 73 of the cup may be an absorbent matrix material, or may, for example be an open mesh of a rigid plastics material. Such meshes may readily be coated with the reagents required for the development step.

Connecting wires 75 and 76 serve to connect electrode 71 and 72 to appropriate current measuring means.

The embodiment illustrated in Figures 13a and 13b is a single-test device, so constructed to enable several similar devices to be clipped together so as to form a multiple-test device. The device of Figure 13a comprises an outer cover 80 formed of water impervious material. The cover 80 is open along its longitudinal edges, and heat sealed along its short edges 83. Formations 84a and 84b enable two or more devices to be clipped together, to form a multiple-test unit. The upper surface of cover 80 is provided with an aperture 81, to receive a cup 82, which clips into aperture 81. Cup 82 has a base 85 formed of a latex-filled paper (L.S.A.S., Milton, Cambridge), and has been previously treated with a labelled conjugate material, usually an antibody conjugated to an enzyme such as alkaline phosphatase. A polystyrene plate 86 rests on the base of envelope 80, such that paper 85 rests in contact with plate 86, when cup 82 is clipped into aperture 81. The space in envelope 80 at either side of aperture 81 is filled with an absorbent wadding material (Schleicher and Schuell pre-filter 2294).

Prior to assembly, the polystyrene of plate 86 is coated with an antibody to a substance for which it is intended to test. In use, a fluid sample containing an antigen under test is applied to filter matrix 85,

in contact with polystyrene plate 86. The addition of the fluid sample dissolves the alkaline phosphatase-antibody conjugate in filter matrix 85, and the conjugate, antigen under test, and antibody bound to plate 86 take part in an immunological reaction, to bind to the plate 86 an amount of conjugate dependent upon the amount of antigen in the sample. Filter matrix 85 retains the sample in contact with polystyrene plate 86. After incubation, cup 82 is removed, and excess conjugate is washed from surface 86 by applying a washing solution, the washing solution being taken up by absorbent pads 88. Cup 82 is then replaced with a similar cup containing developer chemicals. For example, matrix 85 in the similar cup may contain the dry components of a developer comprising NADP or NADPH, as disclosed above.

The developer components cause the development of a coloured product, which enable a rapid yes/no determination to be made of a clinical condition.

Figures 14a to 14c illustrate an alternative construction in accordance with the invention, in which a sample and three calibration areas are present in the same device. The test device comprises an outer casing 100 formed of an lower cover 102 and an upper cover 101, sealed at their edges 103. An aperture 104 is formed in the upper cover 101, to

receive a support 105, in the form of a frame. The support 105 carries four absorbent filter matrix pads, 106, 107, 108 and 109. Frame 105 is a clip-in fit in hole 104. Filter pads 106, 107, 108 and 109 are identical, and each carry, in dry form, an antibody, or DNA fragment, conjugated to an enzyme. In use, pad 106 is used for the sample, and known concentrations of the substance under test are applied to filters 107 and 108. 109 is used as a blank. The specific binding reaction takes place generally as outlined above.

Figures 14b and 14c illustrates an incubation step, in which an absorbent pad 111 contains the enzyme conjugate as outlined above. Pad 111 is supported on a mount 112 of a rigid plastics material, for example polystyrene. The specific binding reaction takes place at the surface of polystyrene strips 114, provided in the base of container 100. Wash wadding 116 is provided as in the device of Figure 13, to enable the polystyrene strips 114 to be easily washed between the incubation and development steps, simply by application of a washing liquid to strips 114.

Figures 15a and 15b illustrate an electrode assembly suitable for use with the device shown in Figure 14a, instead of developer pad 112. The assembly of Figures 15a and 15b comprises a frame 120,

usually of a resilient plastics material, and having a base section 124. Base section 124 may preferably be formed of a copper-clad plastic, of the type used to make printed circuits. Four absorbent pads 121 are mounted on the surface of base 124, each of the absorbent pads being provided with the components of an electro chemical amplifier system, as will be described in detail below. A hole 125 is formed in base part 124 immediately under each pad 121, so that reagents may be added during the development reaction. Beneath each pad 121, two electrodes 122, 123, are formed on the surface of the base 124. One electrode of each pair is formed of a resin-bonded graphite (HY67, produced by Morganite Electrical Carbon Ltd.) and the other is a silver/silver chloride electrode. The electrodes 122, 123 are connected to a connector (not shown), by means of copper tracks 128. In use, the specific binding reaction is carried out using the apparatus shown in Figure 14a. Frame 105 is then removed, and replaced by frame 120. The specific binding reaction may thereafter be measured by measuring the current which passes between electrodes 122, 123, reflecting the amount of enzyme marker bound to the surface of strips 114.

A preferred method and apparatus in accordance with the invention is described in the following example.

EXAMPLE 1

Device for detecting Chlamydia antigen

An opaque white polystyrene solid support (Trade Mark Bextrene-G & W Film Sales Limited) 0.5 mm in thickness was cut into 100 x 30 mm pieces, cleaned with 70% ethanol, and air-dried. The strips were dipped into a 5 microgram/ml solution of a monoclonal anti-Chlamydia antibody, in 200 mm sodium hydrogen carbonate buffer (pH 9.0) with 0.02% thimerosal. The glass tank was pre-treated to avoid antibody preferentially binding to it, by treating with a glazing solution containing 5% lactose monohydrate, 0.5% degraded gelatin, 0.01% thimerosal, 0.05% of a detergent (Trade Mark-Tween 20), and incubating for 4 hours at 37°C. The glazing solution was discarded, and the tank was allowed to drain for two hours prior to the addition of the antibody solution. The polystyrene strips were incubated with the antibody overnight at 37°C.

After removal from the antibody solution, the polystyrene strips were immersed in succession in three beakers containing a glazing solution having the composition noted above. The antibody-coated strips were thereafter dried in air. The strips thus prepared were cut to dimensions of 30 x 10 mm, for fabrication of the device of Figure 14. The absorbent filter matrix 106 of Figure 14 was a latex-filled

paper (L.S.A.S., Milton, Cambridge), which was pre-blocked by immersion for one hour at 37°C in the above glazing solution, and dried in air.

An Fab' cleaved anti-Chlamydia antibody, conjugated to alkaline phosphatase (Ishikawa, E., Kawai, T & Miyai, K. 1981, "Enzyme Immunoassay". Igaku - Shoin. Tokyo) was prepared and diluted to 5 mg/ml in a buffer containing 6.05 g/l Tris, 29.2 g/l sodium chloride, 5 g/l zwitterionic detergent (Trade Mark CALBIOCHEM SB14), 20 g/l bovine serum albumin, 1 ml/l 0.1M zinc chloride, 2 ml/l of a further detergent (Trade Mark TRITON X705), 10 ml/l of a 10 mg/ml pig IgG and 0.05 g/l thimerosal. Forty microlitres of the conjugate solution was pipetted onto each of four paper squares 10 mm^2. The conjugate was freeze dried into the filter matrix.

A washing buffer was prepared which was able to form the dual function of washing the plates 114, and acting as a solvent for the reagents used in the detection of the enzyme label. The washing buffer comprised 75 mM diethanolamine (pH 9.0), 1mM iodonitrotetrazolium violet, 0.02% sodium azide, 50 micromolar EDTA, and 0.5% ethanol.

After the immunological reaction between the immobilised antibody and the sample antigen, takes

place on the impermeable solid support, the support is washed using the above washing buffer, and any labelled antibody bound to the antigen is detected using a developer pad as illustrated in Figure 14b, in which the absorbent matrix 114 comprises, for example, 1.6 mg/ml copper activated pig heart diaphorase, 12.7 mg/ml alcohol dehydrogenase, 2.3 mg/ml sucrose, 3.0 microlitre/ml detergent (Trade Mark TRITON X705-50%), 54.6 mg/ml degraded gelatin, 5mM Tris (pH 7.2). When a sample containing Chlamydia antigen is applied to filter matrix 106, and subsequently developed using the above method, red colour appears on the developer pad 111 within five minutes. The colour development can be halted by the addition of 5 microlitres of 0.015% sulphuric acid.

EXAMPLE 2

Device for detecting follicle stimulating hormone (FSH)

The device was generally similar to that described in Example 1, except that the anti-Chlamydia antibody was replaced with anti-FSH antibody the conjugate employed in pad 106 was an anti-FSH alkaline phosphatase conjugate, employed at a dilution of 0.02 to 5.0 microgram/ml in the buffer used in Example 1.

The same reaction protocol was followed as in

Example 1, and a red colour developed within five minutes.

Example 3

Detection of prostatic acid phosphatase using electrochemical detection in an assay for prostatic acid phosphatase (PAP) was performed in a similar manner to Example 1, but substituting a monoclonal anti-PAP antibody for the anti-chlamydia antibody. The filter paper D203 was pre-blocked as in Example 2, and a 5 microgram/ml non-fragmented anti-PAP antibody conjugated to alkaline phosphatase was freeze-dried into the matrix in a similar buffer to that used in Example 2. The device was tested using standard PAP solutions having a concentration of from 0 to 100 ng/ml in a buffer containing 2.9 g/l trisodium citrate (pH 6), 30 g/l bovine serum albumin, 1 m/l 1M magnesium chloride, 5.8 g/l sodium chloride, 0.1 g/l thimerosal. The procedure was generally as in Example 1. The results were measured electrochemically, using a detection device as shown in Figure 15. In this case, the washing solution contained 10 mM potassium ferricyanide, 200 mM sodium fluoride, 5 mM malic acid (pH 5.0), 4% ethanol, 0.2% sodium azide. The absorbent pads 121 were treated

with a solution containing 0.43 mg/ml copper activated pig heart diaphorase, 2.16 mg/ml alcohol dehydrogenase, 20 micromolar NADP, 100 mM sodium fluoride, 50 micromolar EDTA, 0.42 mg/ml sucrose, 10 mg/ml denatured gelatin, 2-amino-2-methyl-1,3-propandiol (pH 9), 0.7 microlitre/ml of a detergent (Trade Mark TRITON X705-50%), and the pads 121 were then freeze-dried. A 12 second pulse of 650mV was applied between the electrodes, after which the resulting accumulated charge was determined (microcoulombs), and plotted against the concentration of PAP used. The results are shown below:-

ng/ml PAP	charge, microcoulombs
0	11.8
10	21.3
30	36.7
60	55.7
100	73.2

It will of course be understood that although the invention has been specifically exemplifed with reference to immunoassays, the method is equally applicable to DNA hybridisation assays, of generally conventional form or other known forms of biochemical assays.

CLAIMS

1. Apparatus for carrying out a biochemical assay comprising,

a reaction surface adapted to bind a first biochemical ligand,

a liquid absorbent member adjacent the reaction surface adapted to absorb washing solution applied to the reaction surface,

a filter matrix adapted to contain a second biochemical ligand, capable of being specifically bound with the first biochemical ligand, and to retain the said second ligand in contact with the reaction surface to overlie the reaction surface in close contact therewith.

2. Apparatus as claimed in Claim 1, wherein the filter matrix is removeable to facilitate washing of the reaction surface.

3. Apparatus as claimed in Claim 1, wherein the liquid absorbent member is constituted by an area of the material forming the carrier matrix.

4. Apparatus as claimed in Claim 1, wherein the filter matrix comprises a labelled substance capable of a specific-binding reaction at the reaction surface during the assay, to enable a sample material retained in contact with the ligand support by the filter

matrix to be determined, by measurement of the amount of the labelled substance bound to the reaction surface.

5. Apparatus as claimed in Claim 4, wherein the labelled substance is a water-soluble substance, which is present in the filter matrix in a dry form.

6. Apparatus as claimed in Claim 4, wherein the labelled substance is an enzyme, conjugated to a biochemical ligand.

7. Apparatus as claimed in Claim 6, wherein the labelled substance is an enzyme conjugated to an antibody, an antigen, a hapten, or a nucleotide sequence.

8. Apparatus as claimed in Claim 1, wherein the filter matrix is a filter paper.

9. Apparatus as claimed in Claim 1, which includes an additional filter for pre-filtering a sample.

10. Apparatus as claimed in Claim 1, comprising a movable support for at least one developer, the developer being a substance enabling determination of the amount of a label bound to the reaction surface during the biochemical reaction, and wherein the apparatus includes means for retaining the said movable developer support in close proximity with the said reaction surface.

11. Apparatus as claimed in Claim 10, wherein the said movable developer support comprises an absorbent matrix for containing and supporting the developer.

12. Apparatus as claimed in Claim 11, wherein the movable developer support is a cup-like member or frame, of which the said absorbent matrix forms the base.

13. Apparatus as claimed in Claim 10, wherein the assay is an enzyme-labelled assay, and the developer support comprises an enzyme adapted to take part in a colour development reaction.

14. Apparatus as claimed in Claim 1, wherein

(a) the first ligand is a monoclonal antibody or a nucleotide sequence,

(b) the first ligand is a general antibody, and the filter matrix also comprises a monoclonal antibody-containing material, adapted to bind to the general antibody bound to the reaction surface,

(c) the first ligand is an antigen or a hapten, which is adapted to bind specifically with an antibody in the sample, or

(d) the reaction surface is such as to bind glycosylated haemoglobin and thereby enable reaction of a glycosylated haemoglobin with a specific binding protein.

15. A method of carrying out a biochemical assay, which method comprises

providing a first biochemical ligand bound to a non-liquid-retaining reaction surface,

providing an absorbent filter matrix in contact with the reaction surface,

providing a second biochemical ligand absorbed in the matrix, the second biochemical ligand being capable of a specific binding reaction with the first biochemical ligand,

wherein at least one of the first and second biochemical ligands are applied to the filter matrix in the form of a solution and transferred to the said reaction surface and subsequently filtered by capillary action in the said filter matrix,

maintaining the filter matrix in contact with the reaction surface to cause the second biochemical ligand to take part in a specific binding reaction with the first biochemical ligand on the reaction surface, thereby causing the second biochemical ligand to become bound to the reaction surface,

and determining the result of the said assay in accordance with the amount of the second biochemical ligand which is bound to the reaction surface.

16. A method as claimed in Claim 15, wherein the first biochemical ligand is an antigen, an antibody, or a nucleotide sequence, and is provided on the reaction surface prior to commencement of the assay.

17. A method as claimed in Claim 15, wherein the first ligand becomes bound to the reaction surface

during the progress of the assay.

18. A method as claimed in Claim 15, including the step of removing the filter matrix from contact with the reaction surface after the second ligand has become bound to the reaction surface, to facilitate washing of the rection surface.

19. A method as claimed in Claim 15, wherein the filter matrix initially comprises in dry form a third biochemical ligand incorporating a detectable label, and wherein addition of a fluid sample to the filter matrix causes dissolution of the said third biochemical ligand, whereby the said third ligand is able to bind with the said biochemical ligand at the reaction surface.

20. A method as claimed in Claim 15, and including the step of developing a label bound to the said reaction surface during the assay by providing a developer support, incorporating a developer for the said label, and bringing the developer support into close proximity with the reaction surface to enable the developer to react with bound label on the reaction surface.

FIG. 1

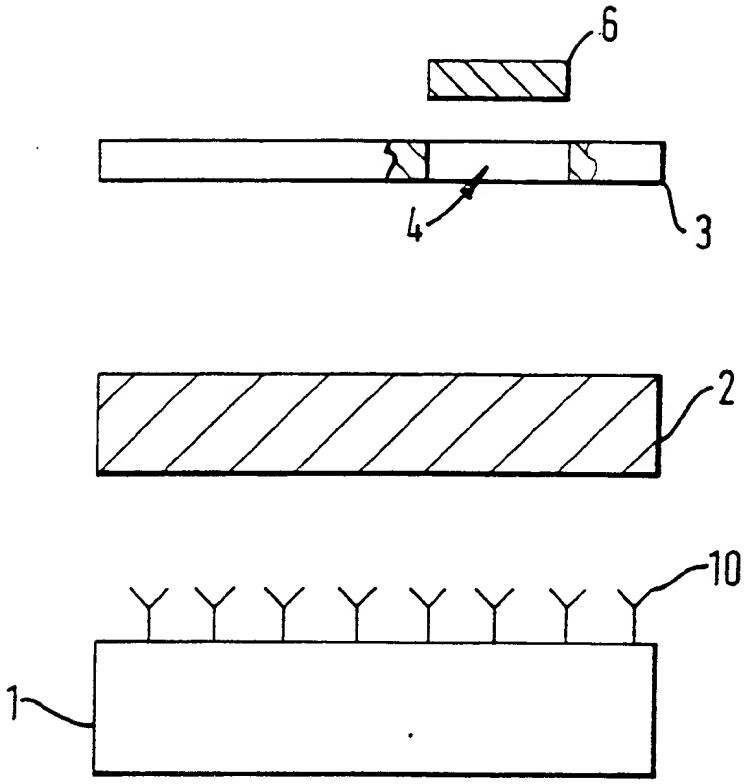


FIG. 2

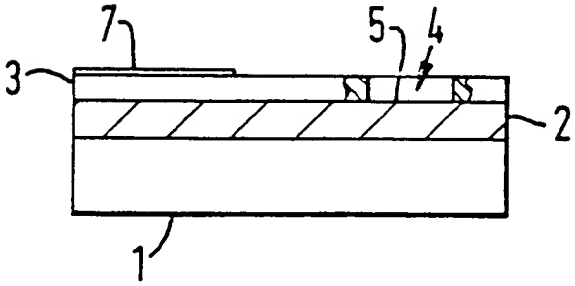


FIG. 3

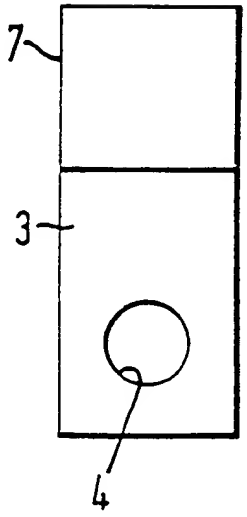


FIG. 4

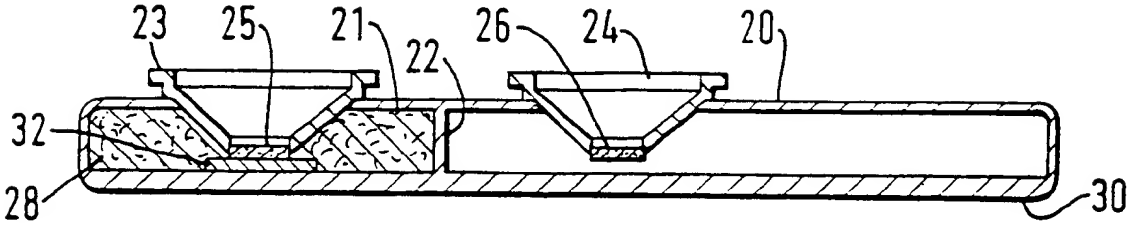


FIG. 5

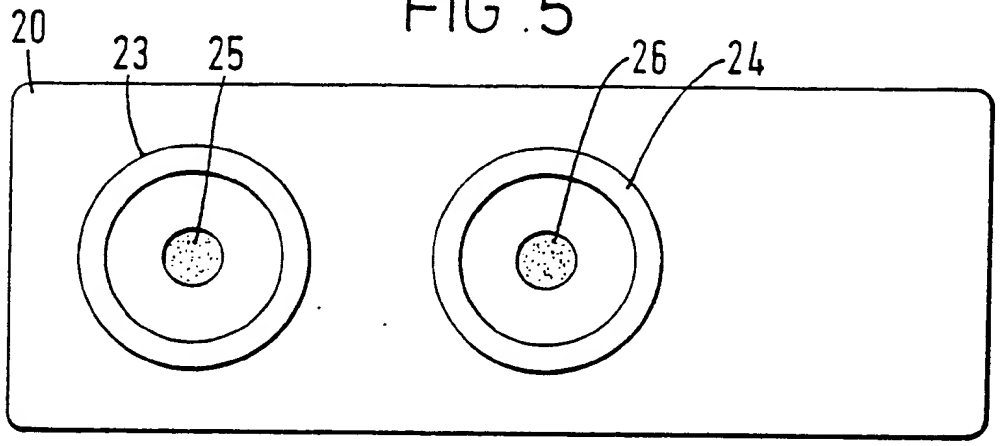


FIG. 7

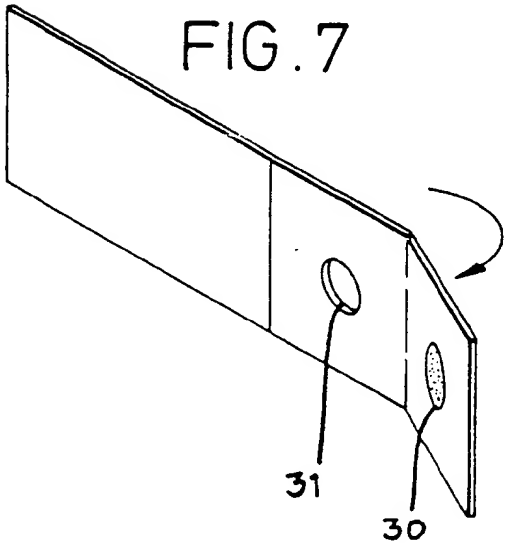


FIG. 8

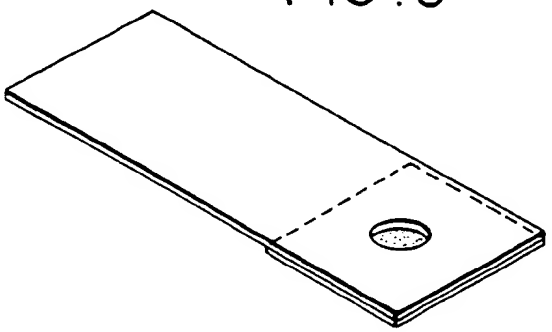
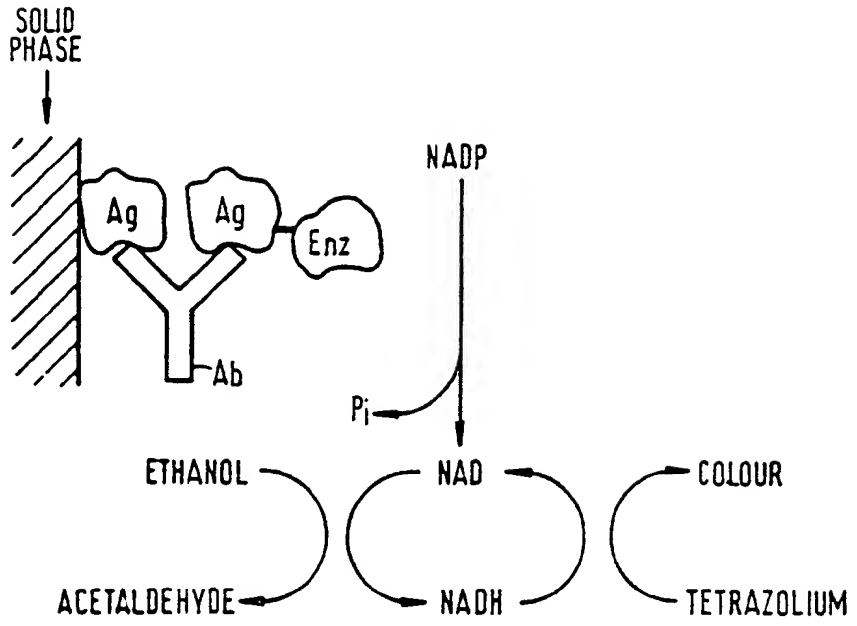


FIG : 6



Ab = ANTIBODY (TO BE DETECTED)

Ag = RUBELLA ANTIGEN

Ag Enz = CONJUGATE OF RUBELLA ANTIGEN AND ALKALINE PHOSPHATASE

FIG . 9

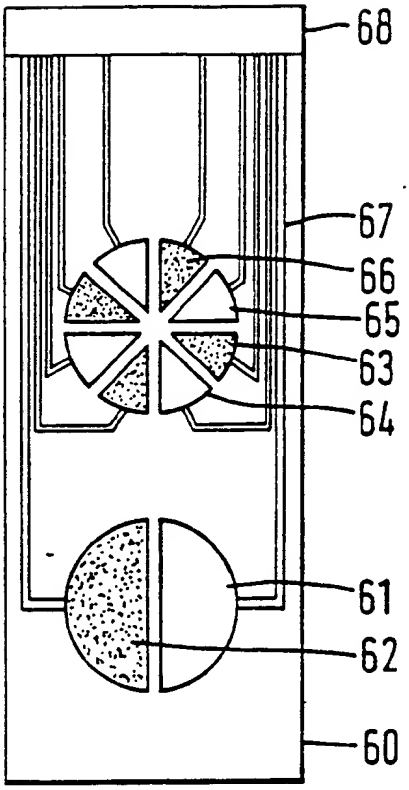


FIG . 10

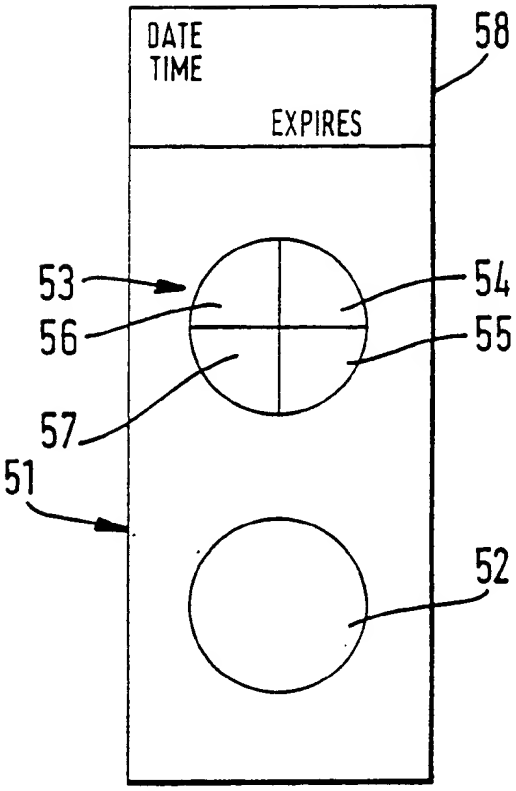


FIG . 11

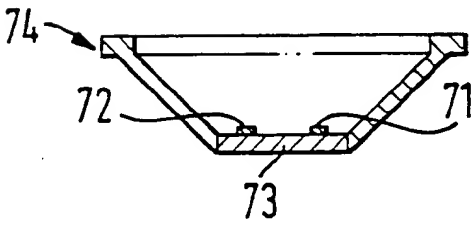


FIG . 12

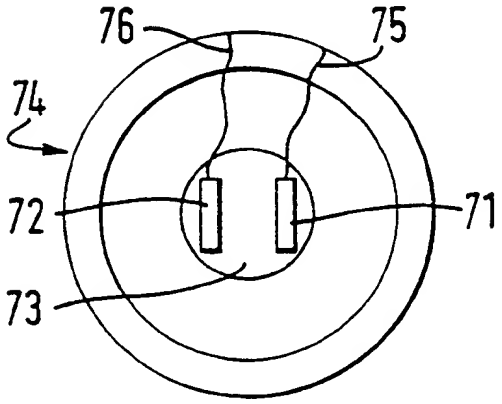


FIG. 13a

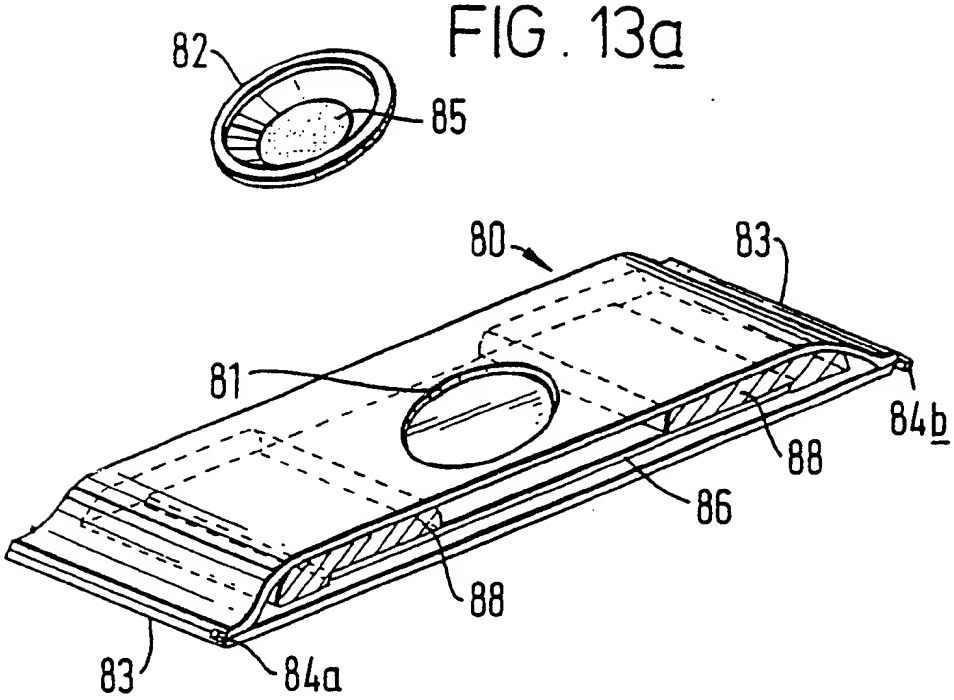


FIG. 13b

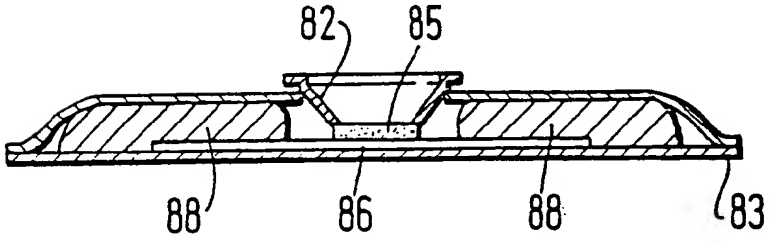


FIG. 14a

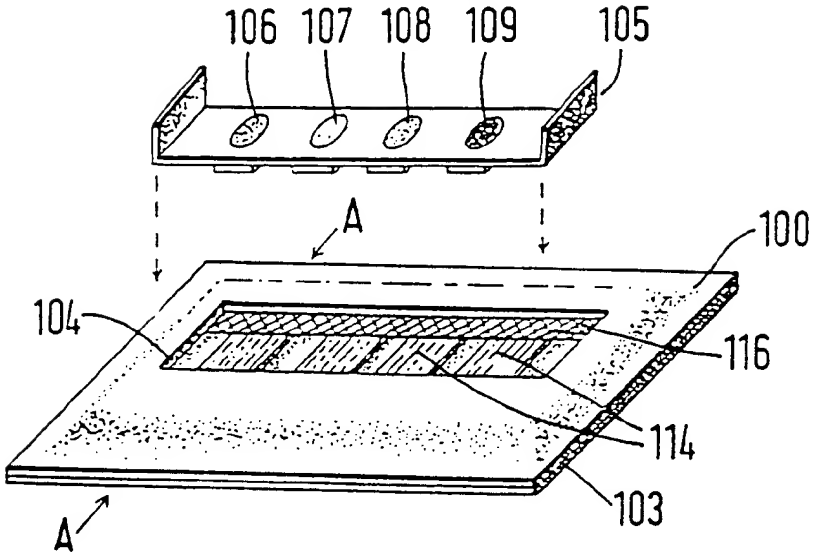
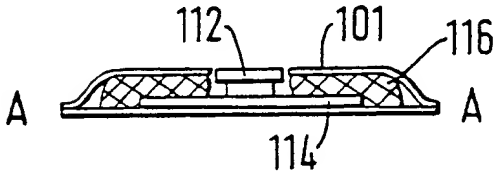


FIG. 14b



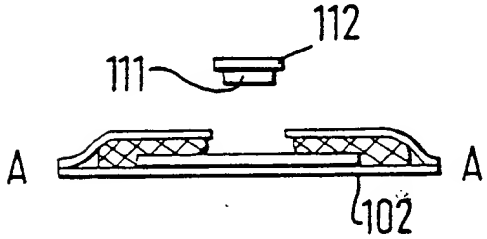


FIG. 14c

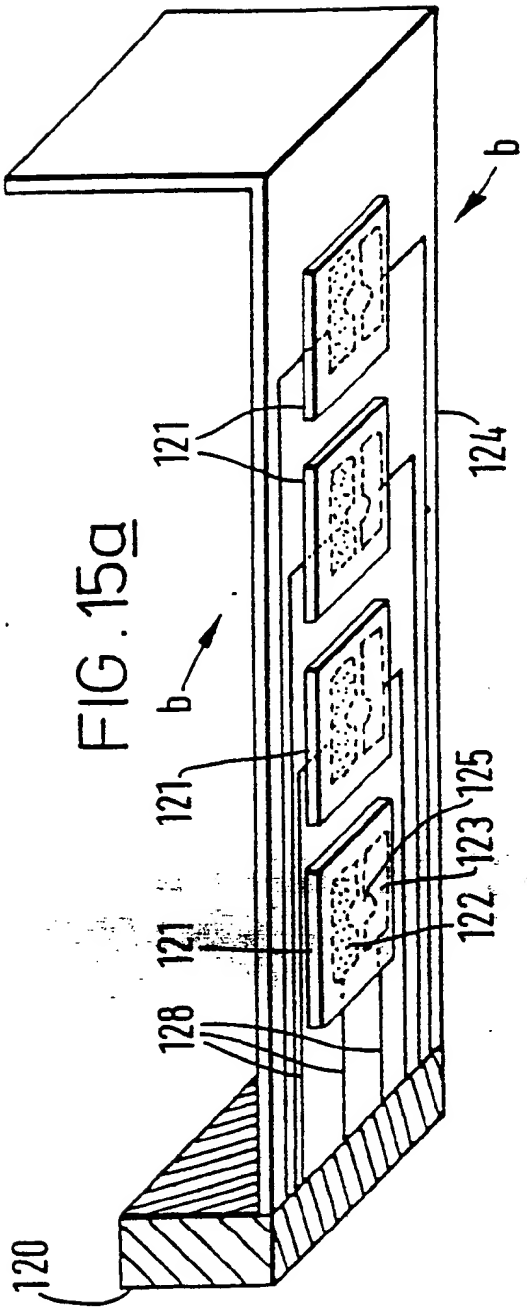


FIG. 15a

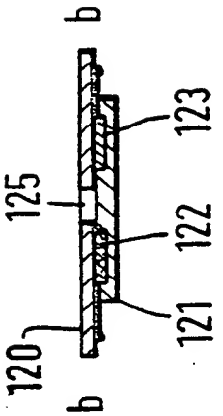


FIG. 15b

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 87/00899

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: G 01 N 33/52

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	WO, A, 80/2077 (GENERAL ELECTRIC CO.) 2 October 1980 see page 3, line 24 - page 6, line 13; page 8, lines 18-22; page 10, line 12 - page 11, line 1; page 13, claims 1,12,14 cited in the application	1,3,12, 14-16
X	US, A, 4459358 (C.M. BERKE) 10 July 1984 see column 3, lines 10-21; column 4, lines 9-49; column 11, line 11 - column 12, line 34	1,3,14-16
X	FR, A, 2514511 (L.A. LIOTTA) 15 April 1983 see page 2, line 9 - page 3, line 11; page 4, line 26 - page 5, line 34; page 7, lines 12-23; page 9, lines 1-9; page 11, "Stade 3"; claims 1-19	1,3-7,13-17,20
X	DE, A, 3130749 (BOEHRINGER MANNHEIM GmbH) 24 February 1983; page 1, lines 1-26; page 5, line 29 - page 6, line 16; page 10, lines 23-33	1-3,10, 11,14,15, 18,20

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
22nd March 1988	15 APR 1988
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: P.C.G. VAN DER PUTTEN

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	US, A, 4256693 (A. KONDON & M. KITAJIMA) 17 March 1981 see column 1, line 62 - column 2, line 9; column 3, lines 51-60; column 4, line 66 - column 5, line 3; column 6, line 64 - column 7, line 5 --	4,8,9,18, 19
X	EP, A, 0197266 (SAGAX INSTRUMENT AB) 15 October 1986 see page 3, lines 1-6; page 4, lines 12-14; page 6, example 1; page 10, lines 1-16	14,16

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8700899 SA 19796

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 06/04/88
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 802077		None	
US-A- 4459358	10-07-84	WO-A- 8505686	19-12-85
		EP-A- 0185654	02-07-86
FR-A- 2514511	15-04-83	DE-A- 3237046	21-04-83
		BE-A- 894662	11-04-83
		SE-A- 8205751	08-10-82
		JP-A- 58076763	09-05-83
		NL-A- 8203946	02-05-83
		LU-A- 84402	13-06-83
		GB-A,B 2111676	06-07-83
		US-A- 4446232	01-05-84
		CA-A- 1200483	11-02-86
DE-A- 3130749	24-02-83	None	
US-A- 4256693	17-03-81	None	
EP-A- 0197266	15-10-86	DE-C- 3506703	30-04-86
		JP-A- 61227591	09-10-86